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**THE ROLE OF
BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF)
ON EPILEPSY**

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Resumo

A epilepsia é habitualmente definida como uma condição de predisposição crónica para convulsões. É de destacar que nenhum dos “fármacos antiepiléticos” utilizados na prática clínica conseguem prevenir o desenvolvimento de epilepsia depois de um estímulo epilético, e por isso são cada vez mais denominados de fármacos anticonvulsivantes em vez de antiepiléticos. Conjuntamente com o facto de que 50% dos pacientes tratados continuarem a experienciar convulsões, as intervenções farmacológicas nesta doença estão ainda longe do desejável, e consequentemente novas estratégias terapêuticas são necessárias. Apesar de controverso, a modulação da sinalização das neurotrofinas, em particular do BDNF tem sido vista como uma estratégia terapêutica promissora.

Esta revisão tem como objetivo compilar a informação disponível relativamente ao papel da sinalização do BDNF na epilepsia, e os possíveis mecanismos envolvidos, tais como a modulação da transmissão neuronal excitatória e inibitória; “mossy fiber sprouting” e o neuropeptídeo Y.

Os dados apresentados demonstram uma clara discrepância entre as observações de vários estudos. Tal facto pode estar relacionado com as diferenças existentes entre: 1) modelos animais; 2) o protocolo de indução de convulsões espontâneas recorrentes; 3) esquema terapêutico de administração do BDNF exógeno; 4) modulação da sinalização em modelos genéticos de BDNF.

Conclui-se então que há necessidade de mais estudos para desvendar o papel desta neurotrofina na epilepsia e para aferir se a modulação das suas vias de sinalização poderá constituir estratégias terapêuticas.

Palavras-Chave

Neurotrofinas; BDNF; TrkB; Epilepsia; recetores A_{2A} da adenosina; NMDA; GABA; NPY

Abstract

Epilepsy is commonly defined as a condition of chronic predisposition for seizures. Importantly, none of the “antiepileptic drugs” used in clinical practice can prevent the development of epilepsy after an epileptic insult, and thus are increasingly referred to as anti-seizure drugs rather than anti-epileptic. Together with the fact that about 50% of treated patients continue to experience seizures, the pharmacological intervention is far from the ideal, and consequently the need for new therapeutic alternatives emerges. Although controversial, the modulation of neurotrophin signaling, in particular of the brain-derived neurotrophic factor (BDNF), has been seen as a promising therapeutic strategy.

The present review aims to compile the available data related to the role of BDNF signaling on epilepsy and the possible mechanisms involved such as modulation of excitatory and inhibitory neuronal transmission; mossy fiber sprouting, and neuropeptide Y.

Taken together, the presented data clearly show a discrepancy among the available data. This could be related to differences in the 1) animal model; 2) induction of spontaneous recurrent seizures protocol; 3) schedule of exogenous BDNF administration; 4) genetic models of BDNF signaling modulation.

More studies are needed to unveil the role of this neurotrophin upon epilepsy and whether the modulation of its signaling pathways could be considered a therapeutic strategy.

Keywords

Neurotrophins; BDNF; TrkB; Epilepsy; A_{2A} of Adenosine; NMDA; GABA; NPY.

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“My best friend is the one who brings out the best in me” - Henry Ford

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“O homem é do tamanho do seu sonho” - Fernando Pessoa

Abbreviations

A_{2A}R: adenosine A_{2A} receptors
A₁R: adenosine A₁ receptors
BDNF: brain derived neurotrophic factor;
Ca²⁺: calcium;
EEG: electroencephalogram;
GABA: gamma-aminobutyric acid;
GFLs: glial cell line-derived neurotrophic factor family of ligands;
K⁺: potassium;
KA: kainate acid;
MFS: mossy-fiber sprouting;
Mg²⁺: magnesium
MMP7: metalloproteinase gene matrilysin;
MTLE: mesial temporal lobe epilepsy;
NGF: nerve growth factor;
NMDA: N-methyl-D-aspartate receptor;
NPY: neuropeptide Y;
NT: neurotrophin;
NT-3: neurotrophin-3;
NT-4/5: neurotrophin-4/5;
NTF: neurotrophic factors;
PDS: paroxysmal depolarizing shift;
SE: status epilepticus;
SRS: spontaneous recurrent seizures;
TrkB-Fc: Tropomyosin receptor kinase B receptor body, scavenger for BDNF
TrkB-FL: Tropomyosin receptor kinase B full-length
TrkB-Tc: Tropomyosin receptor kinase B truncated;

1. Introduction

Epilepsies are frequently devastating disorders, with a prevalence around 1% in the world's population, being the third most common neurologic disorder, after dementia and stroke. There are about 40 distinct forms of epilepsy identified. Although 50% of this cases have a complete control of seizures and a further 25% have improved significantly under medication, remains 25% of patients whose disease has poor control although optimized therapy. Given the heterogeneous clinical presentation of the patients and the different responses to therapy, some authors prefer the designation epilepsies rather than epilepsy

The definition of epilepsy and epileptic seizure have been changing since the 1981 consensus. In 2014 a new consensus defined epileptic seizure as a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain; and epilepsy as a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of this condition, requiring the occurrence of at least one epileptic seizure (Fisher et al., 2014). Conversely, there are “non-epileptic” seizures, when evoked in a normal brain by treatments such as electroshock or chemical convulsants. The therapy for patients with seizure disorders is usually multimodal and it includes the treatment of underlying conditions, avoidance of precipitating factors and suppression of recurrent seizures with medication or surgery. However, whether any of the antiepileptic drugs being used in clinical practice can prevent the development of epilepsy (epileptogenesis) is uncertain, and so drugs are preferentially referred as anti-seizure rather than antiepileptic. Together with the evidence of about 20% of cases resistant to therapy, emerges the need of better therapeutic strategies. Neurotrophins, namely brain derived neurotrophic factor (BDNF) have been implicated in the physiopathology of epilepsy, and an increasing interest for a potential role in its therapeutics has been growing.

2. Neurotrophins

Neurotrophic factors (NTF) are a superfamily of proteins which are known to be essential in neuronal survival, growth and differentiation and to have acute effects on modulation of synaptic transmission and plasticity. Specifically, its effects on mature neuronal circuits include modulation of synaptic efficacy and plasticity as Long-term potentiation (LTP) and Long-term depression (LTD); and its effects on neuronal circuits development include modulation of neural stem cell survival and differentiation, survival of differentiated neurons, axon-dendrite differentiation, growth and guidance; synapse formation and maturation (Huang and Reichardt, 2001).

The NTF superfamily comprises four distinct major groups: (1) the neurotrophin (NT) family; (2) the glial cell line-derived neurotrophic factor family of ligands (GFLs); (3) neurotrophic cytokines (neurokines); and (4) the family of cerebral dopamine neurotrophic factor (CDNF) and mesencephalic astrocyte derived neurotrophic factor (MANF) (Rodrigues et al. 2014).

NT are a closely related group of secreted proteins that are initially synthesized as larger precursors (proneurotrophins), which undergo proteolytic cleavage to yield mature forms. In mammals, the neurotrophin family includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). The biological actions of neurotrophins are mediated either by the activation of their cognate tropomyosin-related kinase receptor (TrkA, TrkB or TrkC), or by the activation of the common p75 neurotrophin receptor (p75NTR). TrkA is the high-affinity receptor for NGF (with low-affinity binding by NT-3 in some systems) (Kaplan et al., 1991; Klein et al., 1991), TrkB is the high-affinity receptor for BDNF with lower-affinity binding by NT-3 and NT-4 (Berkemeier et al., 1991; Squinto et al., 1991), and TrkC is the high-affinity receptor for NT-3 (Lamballe et al., 1991; Urfer et al., 1995).

In addition to the high-affinity NT receptors, all of the NTs bind to the low-affinity NT receptor, designated as p75NTR. (Chao and Hempstead, 1995). The p75NTR is a member of the tumor necrosis factor (TNF) receptor superfamily, and the p75NTR activation elicit distinct cellular responses, inducing cellular death when binding to pro-neurotrophins namely through JNK and NADE pathways (Casaccia-Bonofil et al., 1996; Friedman, 2000), and modulating the affinity and selectivity of Trk activation when binding to mature neurotrophin (Teng et al., 2010). Although p75NTR in the highest levels are observed perinatally, then it declines with age, it is also overexpressed in various pathological conditions, including epilepsies, axotomy and neurodegeneration (Dechant and Barde, 2002).

In this work we review the specific role of the neurotrophin BDNF on epilepsy therefore BDNF signaling will be detailed in this section.

BDNF uncleaved form, the pro-BDNF is a 32-kDa precursor, comprising 247 amino acids with N-glycosylated and glycosulphated residues within the prodomain (Mowla et al., 2001). Following the initial

generation, most of the pro-BDNF is then packaged into vesicles in a regulated pathway and undergoes N-terminal cleavage by extracellular proteases, such as plasmin, metalloproteinase gene matrilysin (MMP7) (Lu, 2003; Pang et al., 2004), tPA/plasmin cascade and extracellular matrix-metalloproteinases (Lee et al., 2001). In trans-Golgi network, the 'pro-region' is cleaved resulting in the formation of mature BDNF (14 kDa), a biologically active form with C-terminal dimers (Seidah et al., 1996). This mature BDNF could be then released by the neurons through constitutive secretion or in an activity dependent manner (Mowla et al., 2001). But although BDNF is highly expressed in neurons, astrocyte and microglia are also capable of producing BDNF (Miklic et al., 2004).

Pro-BDNF selectively activates its high affinity receptor, the p75NTR, mainly inducing pro-apoptotic signaling pathways (Teng et al., 2005) while mature BDNF binds to TrkB receptors and as well as to the low-affinity neurotrophin receptor p75NTR (Lahtinen et al., 2003).

BDNF binding to the full length (FL) receptor TrkB-FL triggers autophosphorylation of the tyrosine residue in its intracellular domain, leading to ligand-induced dimerization in each receptor, which activates several intracellular signaling pathways. The major signaling pathways activated by the TrkB-FL receptors are; (1) PI3K/AKT/mTOR; (2) MAPK/ERK and (3) PLC γ pathways, and their downstream effectors. Conversely, these include PI3K stimulation of AKT, Ras stimulation of mitogen-activated protein MAPK cascades and PLC- γ dependent generation of inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) (Segal and Greenberg, 1996; Arévalo and Wu, 2006). Which one of the pathways leads to different cellular responses, specifically the PI3K/AKT signalling pathway is thought to be the main responsible by chronic effects of BDNF in neuronal circuits, as it is the survival, growth and differentiation. Conversely, it is thought that this pathway can halt apoptosis through phosphorylation and inhibition of pro-apoptotic proteins such as Bad and caspase-9 (Zhou et al., 2000).

3. Epilepsies

Epileptic seizures have been classified into partial seizures, those beginning focally in a cortical site, and generalized seizures, those that involve both hemispheres widely from the outset. Partial seizures account for around 60% of epileptic seizures and are further divided in simple, when there is preservation of consciousness and complex, when consciousness is impaired. Etiology of partial seizures is most commonly secondary to a lesion in some part of the cortex, such as a tumor, developmental malformation, damage due to trauma or stroke, alcohol or drug withdrawal, repeated episodes of metabolic insults, such as hypoglycemia (Berkovic et al., 2006; Longo, 2012). Alternatively, generalized epilepsies have usually a genetic etiology due to inheritance of multiple susceptibility genes, with rare exceptions as it happens with the spontaneous mutations in SCN1A which is responsible for catastrophic severe myoclonic epilepsy of infancy. Generalized epilepsies are usually categorized based on the seizure activity in absence, myoclonic and tonic-clonic seizures (Anon, 1981).

What concerns to the physiopathology, a seizure results of a sudden imbalance between the excitatory and inhibitory forces within the network of cortical neurons in favor of a sudden-onset net excitation (Fisher et al., 2005). When this imbalance happens, a group of neurons starts firing in an abnormal, excessive, and synchronized manner (Hammer and Hammer, 2010) which results in a wave of depolarization known as a paroxysmal depolarizing shift (PDS) (Somjen, 2004).

In a normal brain, there is a protection against PDS once after an excitatory neuron fires it becomes more resistant to firing for a period of time. This is due in part from the effect of inhibitory neurons and the electrical changes within the excitatory neuron. In epilepsy the resistance of excitatory neurons to fire during this period is decreased (Hammer and Hammer, 2010). Conversely, the mechanisms that are generally responsible for the focal-onset seizures are whether a decreased inhibition or an increased excitation of neurons (da Silva et al., 2003; Quyen et al., 2003).

While the mechanisms of decreased inhibition are mainly relate to defective GABAergic system, the mechanisms leading to increased excitation include increased activation of N-methyl-D-aspartate receptor (NMDA) receptors, increased synchrony between neurons due to emphatic interactions and increased synchrony and/or activation due to recurrent excitatory collaterals (Goldberg and Coulter, 2013).

Importantly, neuropathological studies of patients with intractable focal-onset epilepsy have revealed frequent abnormalities in the limbic system, particularly in the hippocampal formation. The most common lesion is hippocampal sclerosis, which is CA1 and CA3 pyramidal neurons loss and end folium sclerosis (loss of neurons within the hilus) with relative sparing of transitional cortex measured at the mid-body of the anterior-posterior axis (Wieser, 2004).

The most prominent hippocampal sclerosis is frequently found in the mesial temporal lobe epilepsy (MTLE) (Rudge et al., 1998) which is the most common form of partial epilepsy in young adults. More subtle

and apparently more common than overt hippocampal sclerosis is granule cell axon (mossy-fiber) sprouting (MFS) (Sutula et al., 1989). The MFS, which create monosynaptic recurrent excitation circuits, consists of single granule cells that bifurcate their MF axons in the hilar region, projecting the collaterals to both the *cornus ammonis* 3 (CA3) region and the molecular layer (ML). As a result some of the granule cells are monosynaptically interconnected (Koyama and Ikegaya, 2004), providing a substrate for positive feedback monosynaptic recurrent excitation (Dudek and Shao, 2004) (figure 1).

Nonetheless, the role of MSF in epileptogenesis is controversial. The classical concept is that a precipitating brain insult trigger transient cascades of events that permanently enhance excitability of neuronal networks through mechanisms including MFS. Recent evidence suggests this permanent changes occur shortly after the insult, and that there is a continuous turnover of sprouted mossy fibers in epileptic patients and animal models. However, reversal of mossy fiber sprouting might not be antiepileptogenic, once blocking its development with rapamycin does not reduce seizure frequency in pilocarpine-treated mice (Buckmaster and Lew, 2011; Heng et al., 2013; Buckmaster, 2014). However rapamycin has side-effects (Swiech et al., 2008), including suppression of axon sprouting by inhibitory GABAergic interneurons and so it is not absolutely clear yet if MFS is just a epiphenomenon unrelated to seizure genesis as some authors recently suggest, or if in fact is part of epileptogenesis.

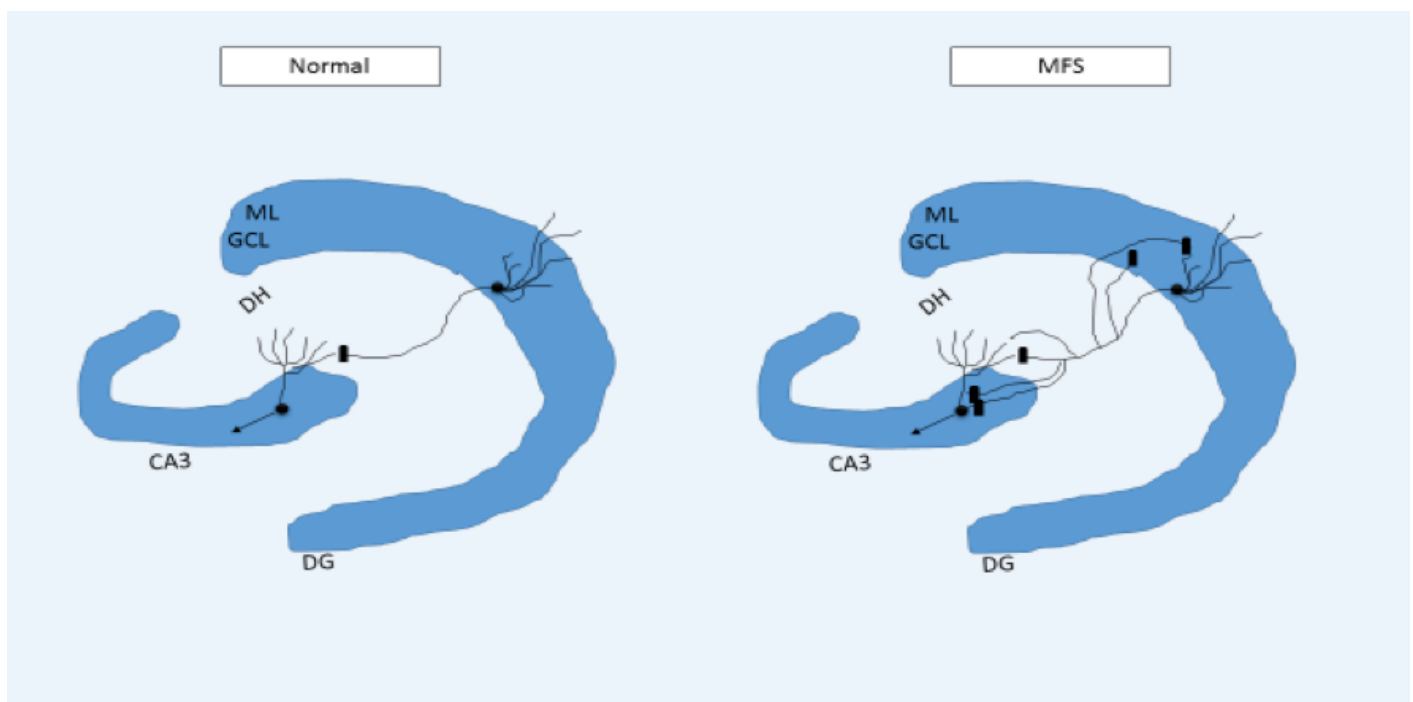


Figure 1 – This image illustrates MFS which occurs in the hippocampus. Neurons with cellular bodies in the GCL bifurcate their axons in the hilar region of dentate gyrus, and project their collaterals o CA3 and to the ML.. Cornus ammonis 3 (CA3); dentate gyrus (DG); dentate hylus (DH); granular cell layer; mossy fiber sprouting (MFS); molecular layer (ML). Adapted from (Koyama and Ikegaya, 2004)

Several animal models have been developed during the last decades and there are indeed various animal models with chronic brain dysfunctions thought to reflect the processes underlying human epilepsy. The most used models to study epilepsy include (i) the kindling model and (ii) post-status epilepticus models. (i) The kindling model is induced by periodic administration of brief, low-intensity electrical stimulation of

the amygdala or other limbic structures. Initial stimulations evoke a brief electrical seizure recorded on the EEG without behavioral change, but repeated (e.g. 10-20) stimulations result in progressive intensification of seizures, culminating in tonic-clonic seizures. Once established, the enhanced sensitivity to electrical stimulation persists for the life of the animal. The limitation of this model is that spontaneous seizures or a truly epileptic condition do not occur until 100-200 stimulations have been administered. In the (ii) post-status epilepticus models, animals experience a prolonged epileptic crisis (status epilepticus (SE)) after injection of pharmacological compounds (e.g. kainate, pilocarpine; intra-cortical penicillin injection; magnesium-free treatment) or via electrical stimulation of (limbic) brain regions. These post-SE models are the most widely used models in epilepsy research today. However, not all aspects of MTLE are reproduced and the widespread brain damage is often a caricature of the situation in the patient. As recently reviewed, more complex mammalian brains and genetic model organisms including zebrafish have been less studied but offer substantial advantages that are becoming widely recognized (Grone and Baraban, 2015).

These models allow exploring the three main intervention levels 1) anti-epileptogenic (prevention of development of transformation of the normal neuronal network into one that is chronically hyperexcitable (Lowenstein, 2015)), 2) anti-seizure (reduction of frequency and/or severity of seizures), and 3) disease-modifying strategies (alteration of the natural history of the disease).

- | |
|--|
| <ul style="list-style-type: none">✓ The most used epilepsy models are kindling and post-SE (pilocarpine and kainate acid) models✓ Three main interventions: anti-epileptogenic, anti-seizure, disease modifying |
|--|

4. Endogenous BDNF and its receptors in epilepsy and in epilepsy models

Seizures have been shown to stimulate the expression of a variety of genes, including transcription factors (Morgan and Curran, 1991; Kiessling and Gass, 1993), neuropeptides (Gall et al., 1991), proteases (Qian et al., 1993) and also neurotrophins (NT) (Gall, 1993).

What concerns to the regulation of the levels of NTs and specifically of BDNF in the epileptic brain, in human, the evidence support they are increased in several cases. Accordingly, in resected hippocampal specimens from patients suffering from TLE compared to control there is an increased expression of BDNF protein (Takahashi et al., 1999) and mRNA (Mathern et al., 1997; Murray et al., 2000). Furthermore, resected specimens from the hippocampus of MTLE patients show an increased NTs expression in brain areas with MFS. Specifically, MFS correlates with increased granular layer nerve growth factor (NGF) immunoreactivity and seizure frequency (Kandratavicius et al., 2013). Similarly, glioneuronal tumors correlated with medically chronically intractable epilepsy also express increased BDNF protein expression (Villemure, 1996).

Recently it has been discussed the relevance of serum BDNF measurement in epileptic patients for a prognostic purpose. A recent study with 135 participants suggests that seizure frequency and epilepsy duration might be negatively correlated with serum BDNF levels independently of other factors (Hong et al., 2014).

Regarding TrkB receptors, western-blot analysis of human epileptic neocortex samples revealed that they are upregulated compared with control patients (Wyneken et al., 2003). However, it is not specified in this study what type of receptors were evaluated, the TrkB-FL or its truncated forms.

In Human:

- ✓ **BDNF mRNA and protein are increased in TLE patients and glioneural tumors associated with epilepsy**
- ✓ **TrkB receptors are upregulated in human epileptic neocortex**
- ✓ **BDNF serum levels might negatively correlate with seizure frequency and epileptic duration**

In animal model of epilepsies induced by kainate acid (KA) administration, it was shown that BDNF mRNA levels gradually increase in dentate granule cells of mice from 24h of status epilepticus onwards reaching a maximum in 7 days (Suzuki et al., 1995; Heinrich et al., 2011). Remarkably, the same evidence was observed performing an electroencephalogram (EEG) analysis that shows spontaneous recurrent discharges progressively developing in the mouse hippocampus during the first two weeks following KA treatment, within the same time-frame as the BDNF increase in dentate granule cells (Heinrich et al., 2011). Accordingly, in the kindling model, it has also been shown an increase in BDNF mRNA expression levels that occur predominantly in the hippocampus bilateral granule cell layer of the dentate gyrus 1-4h after kindled seizure in rat, and the maximum expression levels of BDNF mRNA occurs 2h after seizures (Lindvall et al.,

1994; Morimoto et al., 1998; Binder et al., 2001). Moreover, adult rats treated acutely with peripheral KA revealed that despite robust elevation in both mRNA and protein in multiple brain regions, in the same time frame plasma BDNF is unchanged and cerebrospinal fluid (CSF) BDNF levels remain undetectable (Lanz et al., 2012).

In KA model:

- ✓ **BDNF mRNA and protein expression levels are increased in a way directly correlated with spontaneous recurrent discharges on EEG.**
- ✓ **The increased brain levels of BDNF do not correlate with changes in the CSF or plasma**

Interestingly, no evidences of epileptogenesis were detected in TrkB(-/-) mice even after 50 electrical focal stimulation of the amygdale. However, when exposed to electroshock they exhibit tonic-clonic an clonic seizure similar to wild-type controls. Interestingly, the available data revealed that the duration of seizure progressively increases with focal stimulations of the amygdala in TrkB (+/-) and TrkB (+/+) but not in TrkB (-/-) animals (He et al., 2004), which advances TrkB and its downstream signaling pathways as attractive targets for developing small molecule inhibitors for prevention of epileptogenesis.

Interestingly in BDNF knockout mice, which lack the most important endogenous ligand for TrkB-FL, the BDNF, there is still the promotion of epileptogenesis. This finding could be seen as a contradiction however, this might be explained by the activation of TrkB-FL receptors by other endogenous ligands such as NT-3 (He et al., 2004) and adenosine (Lee and Chao, 2001). And, indeed it was observed that BDNF knockout mice has an increased expression levels of NT-3 in the hippocampus which might contribute to TrkB-FL activation, promoting epileptogenesis progression even in the absence of BDNF (He et al., 2004). Conversely, in kindling model, NT-3 mRNA expression levels are usually decreased (Isackson et al., 1991; Rocamora et al., 1992; Kokaia et al., 1993; Suzuki et al., 1995; Mudo et al., 1996; Gall et al., 1997; Garcia et al., 1997) and it is also reported that the development of amygdala kindling is markedly retarded in mice heterozygous for a deletion of the NT-3 gene. These mice did not reach the fully kindled state (3rd grade 5 seizure) until after 28 +/- 4 days of stimulation compared to 17 +/- 2 days in the wild-type animals (Elmer et al., 1997).

In kindling:

- ✓ **BDNF mRNA and protein are increased in hippocampus**
- ✓ **TrkB gene knockout prevents epileptogenesis in kindling model**
- ✓ **BDNF gene knockout only impairs epileptogenesis in kindling model**

Curiously, a great upregulation of truncated (Tc) TrkB and a decrease in TrkB-FL was observed in a model of epileptic seizures induced by magnesium-free treatment. In this experiment although calpain inhibitors have prevented the decrease in TrkB-FL receptors levels, the impairment on TrkB-FL mediated signaling pathways was not recovered (Xie et al., 2014). This fact was explained by the increased synthesis of TrkB-Tc which is known to act as a negative dominant receptor (Eide et al., 1996). This might constitute a protection mechanism against the possible pro-epileptogenic effect of TrkB-FL signaling pathway activation.

In Mg^{2+} free model:

- ✓ **TrkB-FL is decreased and truncated TrkB is increased compared to control.**
- ✓ **Truncated TrkB increase is mainly due to alternative splicing of TrkB mRNA.**
- ✓ **BDNF signaling is impaired in the presence of TrkB-T1.**

4.1. From seizure to an increase in BDNF levels

What concerns to the seizure initiating mechanism, the bursting activity in individual neurons is caused by a relatively long-lasting depolarization of the neuronal membrane due to influx of extracellular calcium (Ca^{2+}), and generation of repetitive action potentials. With sufficient activation, there is a recruitment of surrounding neurons via a number of synaptic and non-synaptic mechanisms, including: (1) an increase in extracellular potassium (K^+), which blunts hyperpolarization and depolarizes neighboring neurons; (2) accumulation of Ca^{2+} in presynaptic terminals, leading to enhanced neurotransmitter release; (3) depolarization-induced activation of the NMDA subtype of the excitatory amino acid receptor, which causes additional Ca^{2+} influx and neuronal activation (Lowenstein, 2015)

The seizures being responsible for depolarization-induced activation of the synaptic NMDA subtype of the excitatory amino acid receptor results in an influx in Ca^{2+} leading to the immediate release of BDNF which in turn binds to and activates its high-affinity receptor, TrkB-FL (Marini et al., 2007). Moreover the high-frequency activity seen in seizures results in tissue plasminogen activator secretion, inducing the conversion of pro-BDNF to mature BDNF (Boutillier et al., 2008) (Figure 2).

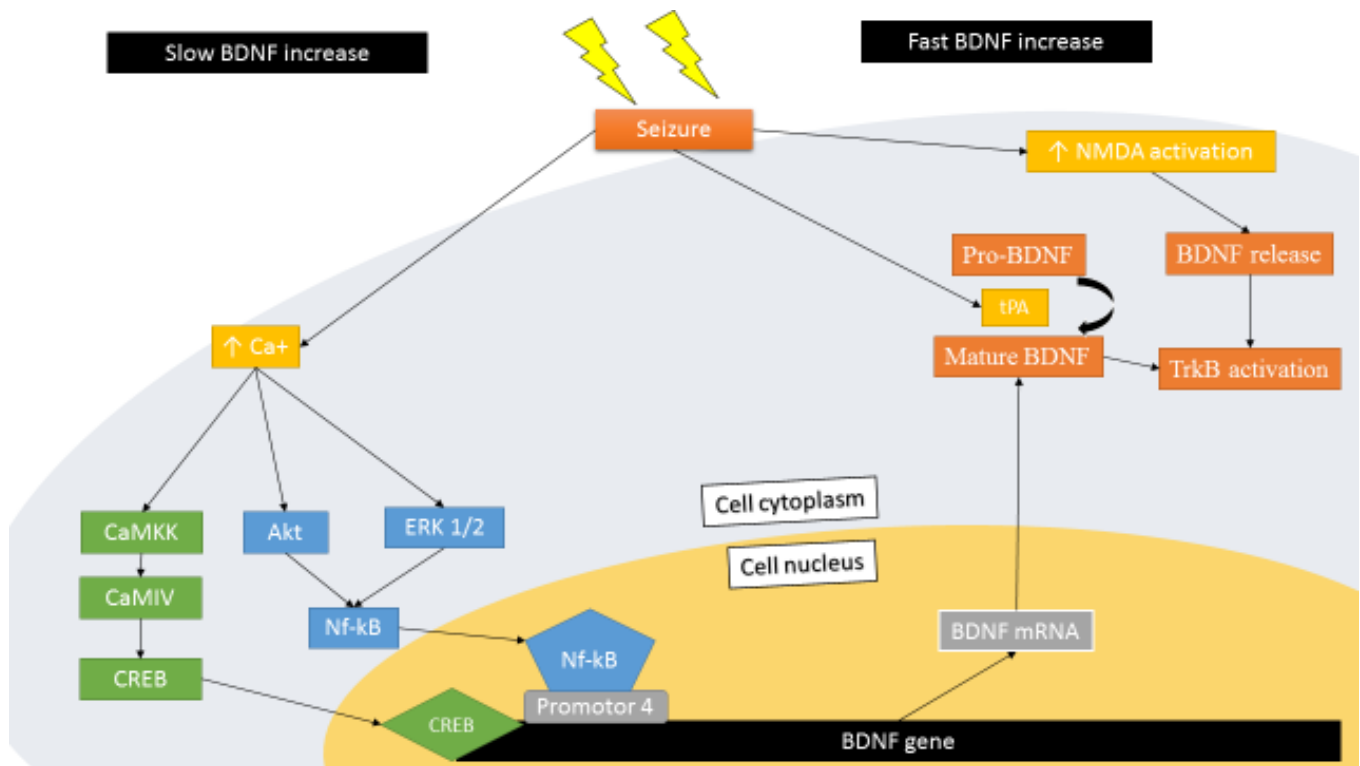


Figure 2 - Seizures lead to a fast and slow increase in BDNF. The Fast increase is dependent on: i) NMDA receptors activation leading directly to BDNF release; ii) conversion of pro-BDNF to mature BDNF through tPA. The slow increase of BDNF is dependent of an increase in intracellular calcium which further increases transcription of BDNF in an AKT/NF-kB and CREB dependent manner. Tissue plasminogen activator (tPA).

Additionally, the influx of Ca^{2+} when NMDA receptor is activated leads to the activation of NF-kB through the AKT and ERK 1/2 pathways. NF-kB is a major survival transcriptional factor (Glazner et al., 2000), that also plays an important role in learning and memory (Meffert et al., 2003). Activated NF-kB translocates to the nucleus where binds to promoter 4 of the BDNF gene increasing its transcription (Lipsky et al., 2001; Marini et al., 2007). The influx of Ca^{2+} also leads to the phosphorylation of cAMP response element-binding protein (CREB) in the nucleus which also binds to and activates promoter 4 of BDNF gene (Tao et al., 1998) (Figure 2).

4.2. From increased BDNF signaling to seizures

As a consequence of BDNF upregulation, there is an enhancement of fast excitatory transmission in pyramidal cells and dentate granule cells of adult rat hippocampus (Kang and Schuman, 1995; Levine et al., 1995; Tanaka et al., 1997), resulting in an increase of neuronal excitability in hippocampal networks, promoting the occurrence of epileptiform activities (Thoenen, 1995). It has already been shown that BDNF potentiates synaptic transmission *ex vivo* (Lohof et al., 1993) and *in vivo* (Messaoudi et al., 1998). It has also been showed that *ex vivo* rat hippocampal slices showed hyper-excitability after acute exposure to BDNF that it is blocked by the inhibitor of Trk receptor phosphorylation, K252a (Scharfman, 1997). Recent evidence also

shows that in a rat model of massive neuronal activation upon kainate-induced seizures it was found that elevated neuronal expression of BDNF is associated with its detachment from the nuclear lamina, and translocation toward the nucleus center. In contrast, the position of stably expressed TrkB-FL remains unchanged after seizures (Walczak et al., 2013). Thus BDNF may interfere with gene expression after seizures.

Also of note is that hippocampal epileptogenesis may develop as a continuous, dynamic and self-aggravating process, in which an initial insult promotes the increase in BDNF expression, that promotes neuronal excitability, including MF sprouting, leading to recurrence of epileptic activities which in turn further increase BDNF expression in the hippocampus (Heinrich et al., 2011).

4.2.1. BDNF influence on neuronal excitability

As stated above, the basic mechanisms responsible for a seizure are reduction of inhibitory synaptic activity or enhancement of excitatory synaptic activity. Consequently, antagonists of the GABAA receptor or agonists of different glutamate-receptor subtypes (NMDA, AMPA, or kainic acid) trigger seizures in experimental animals in vivo. Additional mechanisms leading to increased excitation include increased synchrony between neurons due to emphatic interactions and/or activation due to recurrent excitatory collaterals (Goldberg and Coulter, 2013). There is increasing evidence of BDNF role in some of this mechanisms.

Increased activation of NMDA receptors

BDNF through a mechanism dependent of intraneuronal Ca^{2+} levels increase, facilitates glutamatergic synaptic transmission, mediated by increasing the efficacy of glutamate release from the presynaptic neuron (Drube et al., 1994; Leßmann and Heumann, 1998). Furthermore, BDNF dependent postsynaptic enhancement of NMDA receptor responsiveness has also been shown (Leßmann, 1998). BDNF enhances glutamate release (Canas et al., 2004) and increases activation (Levine et al., 1998) and phosphorylation of glutamate NMDA receptors (Suen et al., 1997) in the hippocampus.

GABAergic transmission

BDNF induce modulation of inhibitory transmission, and the most affected system is the GABAergic. Gamma-aminobutyric acid (GABA) type A receptor (GABA_A Rs) mediate most fast synaptic inhibition in the forebrain. These receptors are constitute by different subunits, and it was observed that alteration in GABA_A R subunit composition occur during epileptogenesis in animal models of epilepsy (Brooks-Kayal et al., 1998).

(1) BDNF activates early growth response factor (Egr3) synthesis via a protein kinase C (PKC)/mitogen-activated protein kinase (MAPK)-dependent pathway, leading to an increase in GABA_AR containing α -4 subunit (Roberts et al., 2006; Lund et al., 2008) (Figure 3). Interestingly, after pilocarpine induced SE, GABA_AR α -4 subunit mRNA expression increases as the animal develops spontaneous recurrent seizures (Brooks-Kayal et al., 1998, 2001). Moreover α -4 containing GABA_AR have been shown to desensitize rapidly (Lagrange et al., 2007). Additionally, they are very sensitive to zinc blockade (Buhl et al., 1996), which is physiopathology important once zinc containing mossy fibers terminals sprout from the granule cells and into CA3, likely depositing zinc onto newly formed α -4 containing GABA_AR, causing a decrease response to GABA.

(2) BDNF activates the JAK/STAT pathway through TrkB-FL receptors activation, increasing inducible cAMP early repressor (ICER), a member of cAMP responsive element-binding protein (CREB) family which lead to a decrease in GABA_AR containing α -1 subunit in *in vivo* model (Lund et al., 2008) (Figure 3). Interestingly, after pilocarpine induced SE, GABA_AR α -1 mRNA expression also decreases (Brooks-Kayal et al., 1998, 2001). Given this evidence, one could advance that the blockade of JAK/STAT pathway would lead to a decrease in ICER, an increase in GABA α 1R and a consequent decrease in seizures. However, it was shown that mutant mice lacking ICER have accelerated kindling (Kojima et al., 2008), developing more severe epilepsy following pilocarpine-induced SE (Porter et al., 2008). Reciprocally, ICER-overexpressing mice show delayed kindling acquisition (Kojima et al., 2008).

This results must be integrated with the multiple signaling pathways of JAK/STAT and ICER. As discussed above, ICER is responsible for increasing BDNF mRNA synthesis, which role in epileptogenesis is not only dependent in GABAergic neurons. Consequently, therapeutic strategy's regarding GABA neurons subunits must aim more specific targets than CREB/ICER.

Moreover, in laboratorial models BDNF was also shown to influence GABAergic neurons in other ways, namely inhibiting GABA release from hippocampal synaptosomes (Canas et al., 2004) and enhancing its uptake from synaptic and extrasynaptic sites and this way influencing neuronal excitability. This uptake facilitation is mediated by GAT-1 molecules at the plasma membrane of astrocytes, which time span is increased as a consequence of BDNF presence in cultures of neurons (Vaz et al., 2011), opposite to what happens in nerve endings, in which GABA uptake seems to be impaired as a consequence of BDNF (Vaz et al., 2008).

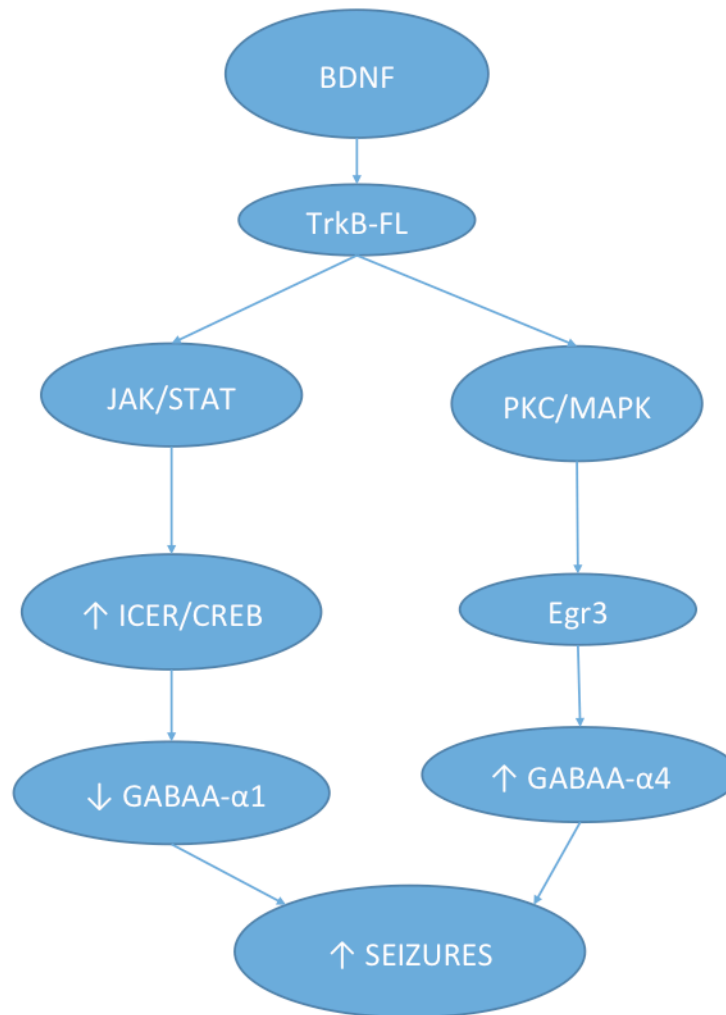


Figure 3 – BDNF may increase seizures through TrkB receptor signaling pathway. TrkB activation diminishes the abundance of the $\alpha 1$ subunit of GABA and increases the abundance of $\alpha 4$ subunit in GABA receptors. Adapted from (Grabenstatter et al., 2012)

- ✓ **BDNF modulates GABA_AR subunits, increasing $\alpha 4$ and decreasing $\alpha 1$ in epileptic animal models**
- ✓ **Mutant mice lacking ICER have accelerated kindling, and ICER overexpressing mice show delayed kindling acquisition.**
- ✓ **BDNF inhibits GABA release from hippocampal synaptosomes, inhibits its uptake by synaptosomes and increases its uptake by astrocytes.**

BDNF mediates mossy fibers sprouting

The involvement of BDNF in MFS can be analyzed in two different perspectives: **(1)** the role of BDNF in developing MFS and **(2)** the influence of BDNF on excitability once MFS is installed.

(1) Role of BDNF in developing MFS

There are complicated, apparently contradictory observations that might be attributed to different experimental procedures or different animal models.

There is evidence that MFS is diminished in mice heterozygous for a deletion of the BDNF gene, in which BDNF expression level is substantially reduced (Vaidya et al., 1999); however, there is also a reported increase on MFS in the same mutant mice (Kokaia et al., 1995b).

There are also reports showing that infusion of BDNF into the hippocampus induces MFS (Scharfman et al., 2002) on contrary there are data revealing that BDNF infusion has no significant effect on MFS (Vaidya et al., 1999). In addition, the scavenger for BDNF, TrkB-Fc, failed to inhibit kainite-induced mossy fiber sprouting in hippocampal cultures (Routbort and Ryan, 1997). Moreover, transgenic mice overexpressing BDNF do not display significant MFS (Qiao et al., 2001). On the opposite, dispersed cultures of granule cells of rat hippocampus showed that MFS can be induced by locally implanted beads of BDNF and prevented with the coapplication of an inhibitor of TrkB phosphorylation or a functioning-blocking anti-BDNF antibody (Koyama et al., 2004).

In this context a suggested interpretation is that MFS is essentially a local phenomenon that is subject to systemic and extrahippocampal influences and that local BDNF action in the dentate hilus trigger MFS, but global application of BDNF mask this effect (Koyama et al., 2004), and this may explain the inconsistency in the literature.

(2) influence of BDNF on excitability upon MFS

MF contains high levels of BDNF protein under basal conditions, and this level of BDNF undergoes further up-regulation after seizure onsets, in KA epilepsy model (Rudge et al., 1998).

Electron microscopy experiments indicate that the vast majority of newly formed MF synapses terminates on dendritic spines of granule cells and that on average one MF forms >500 new synapses and only a minority (<25) contact GABAergic interneurons, being the greatest influence on excitatory neurons (Buckmaster et al., 2002). Given that BDNF facilitates excitatory transmission this can be a major contribution to excitability in epilepsy.

On the other hand, in the healthy hippocampus, more than 90% of MF synapses terminate on GABAergic neurons (Acsády et al., 1998), being MF capable of releasing either glutamate or GABA to synaptic cleft, and peculiarly an increase in GABA expression is observed with seizure activity (Gutiérrez and Heinemann, 2001; Gutiérrez, 2002). However, as already discussed GABA neurons activity is decreased, which might be responsible for increased excitability.

Although the possible deleterious effects of BDNF in the epileptic brain already detailed in this review, robust evidence also support its beneficial effects in some epileptic models, particularly when administered for long periods of time and at low dosage. One of the evidences showing a protective role of BDNF is thought to be mediated by the regulation of neuropeptide Y (NPY) levels.

There are evidences that correlates the levels of BDNF with the levels of NPY: (1) the level of NPY is increased in the neocortex of TLE patients and positively correlated with the BDNF level (Takahashi et al., 1999); (2) In rat epilepsy models, BDNF up-regulation in the MF pathway is followed by an increase in NPY in the same brain region (Vezzani et al., 1999); (3) intrahippocampal infusion of BDNF for seven days induces NPY up-regulation one week after the end of the infusion (Reibel et al., 2000), a time course similar to the time course for the effect of BDNF on kindling model.

There is also robust evidence of the neuroprotector effects of NPY in epilepsy models and seizure control. Accordingly, mice lacking the NPY gene experience spontaneous seizures and are more susceptible to chemically induced seizures as compared to wild-type littermates (Erickson et al., 1996). In addition, transgenic rats overexpressing NPY show lower seizure susceptibility (Vezzani et al., 2002). Moreover, epileptic rats, exhibiting unprovoked spontaneous seizures, significantly lowered seizure frequency and exhibited delayed progression of spontaneous seizure severity following NPY overexpression (Noe et al., 2008).

5. Modulation of BDNF signaling as a therapeutic strategy

We aimed to group the experiments by BDNF modulation schedule in anti-seizure or anti-epileptogenesis properties (Simonato, 2014). However none of the experiments analyzed matched the anti-seizure criteria already defined. Consequently, only the anti-epileptogenesis properties of BDNF were evaluated because in all experiments BDNF modulation was performed before the epileptic model develops SRS.

Epileptogenesis is the process by which the previously normal brain is functionally altered and biased towards the generation of the abnormal electrical activity that subserves chronic seizures (Goldberg and Coulter, 2013). The available data show contradictory results. Tables 1 and 2 summarize the available information concerning the actions of BDNF upon epileptogenesis.

Epileptogenesis was considered whenever there were alterations in relation to control group of: 1) seizure stage; 2) rate of kindling development; 3) SRS either frequency or severity; 4) electrophysiological measurements in EEG; 5) MFS;

In summary there the available data show that 1) decreased levels of BDNF lead to an inhibition of epileptogenesis (table 1) while there are other that 2) demonstrate that increased levels of BDNF inhibits or increases epileptogenesis (table 2). The discrepant data collected might be due to the 1) heterogeneity of the animals used (rat vs mice); 2) heterogeneity of epileptic model (post-SE induced by kainate vs pilocarpine vs penicillin vs kindling model) and even for the same model are different protocols of induction; 3) heterogeneity of the modulation of BDNF levels (e.g. viral vector BDNF supplementation vs direct cannula administration in the brain); 4) different outcomes measured, sometimes only described as reduction of epileptogenesis without further description in the original article.

1) Decrease in BDNF levels lead to an inhibition of epileptogenesis. This conclusion arose from studies where the modulation of BDNF was conducted by several ways: by the knocking down of the BDNF gene (Kokaia et al., 1995a; He et al., 2004; Abidin et al., 2011); by the chronic intracerebro-ventricular or by the local acute administration of the BDNF scavenger, the TrkB-Fc (Binder et al., 1999; Unsain et al., 2009); In all this conditions there was evidence supporting a reduction in epileptogenesis.

2) Increase in BDNF levels might either inhibit or increase epileptogenesis. There are contradictory results depending on the used models: i) different models of genetic overexpression of BDNF consistently show appearance of spontaneous seizures (Papaleo et al., 2011; Isgor et al., 2015) and/or increase of severity of seizures in response to an epileptic stimulus (KA) (Croll et al., 1999). This are particularly appealing results once seems that BDNF overexpression *per si* triggers SRS; ii) Viral vectors to supplement the brain with BDNF and FGF-2 (Paradiso et al., 2009; Bovolenta et al., 2010; Simonato and Zucchini, 2010) as well as

continuous administration through transplanted BDNF-secreting cells (Kuramoto et al., 2011) consistently inhibits epileptogenesis and reduce the frequency and severity of SRS compared to control. This apparently contradictory difference to genetic overexpression is an interesting object of study for which we could not find any consistent explanation in the literature; iii) direct administration of BDNF into the brain retrieved the most interesting results. Continuous intra-hippocampal infusion of BDNF have been shown to inhibit (Xu et al., 2004) and to potentiate (Scharfman et al., 2002) epileptogenesis. However, the same author also observed that multiple bolus injections in spite of continuous administration potentiates epileptogenesis (Xu et al., 2004). It was also observed that direct BDNF infusion to hippocampal slices in electrophysiological studies lead to increased hyperexcitability (Scharfman, 1997).

A possible interpretation of this results is that genetic overexpression of BDNF is related with increased epileptogenesis while BDNF gene manipulation (+/-) and (-/-) is related with the inhibition of epileptogenesis.

The effects of BDNF supplementation in epileptic brain tissue remains relatively unclear, and contradictory results persist. Viral vector localized supplementation with BDNF show interesting results and might constitute a promising therapeutic strategy. However, better comprehension of the mechanism inherent to its anti-epileptogenic properties and possible side effects are needed prior to any clinical trial.

Also remarkable is the observation that administration of BDNF in continuous intra-hippocampal infusion produces distinct results from the ones obtained by the administration in multiple bolus microinjections (Xu et al., 2004). Together with the observation that in BDNF (-/-) mice only modest impairment of epileptogenesis is observed while in TrkB (-/-) mice epileptogenesis is eliminated (He et al., 2004), one might hypothesize that continuous administration of BDNF might downregulate the TrkB-FL receptors, justifying the inhibition of epileptogenesis.

Table 1 - Studies suggesting that BDNF modulation inhibits epileptogenesis.

BDNF levels	Strategies to modulate BDNF levels	Schedule of BDNF modulation	Model	Main observations	References
↑	Transplanted BDNF-secreting cells genetically manipulated	7 days before SE	Rats, in vivo, i.p. injected with KA 45 mg/kg;	Significant amelioration of seizure stage and reduction of the number of abnormal spikes at 7 days after KA administration. Enhanced neurogenesis	(Kuramoto et al., 2011)
	Continuous intra-hippocampal infusion vs multiple bolus microinjections	Microinjection: day 1, 5 and 9; and; infusions 14 days after first electrical stimulation;	Rats, in vivo, kindling model: pulses at a frequency of 60 Hz and intensity 500 to 700A; twice daily for 11 days	Continuous infusion of BDNF caused inhibition of the development of seizures and decreased the level of phosphorylated TrkB receptors; In contrast, multiple bolus microinjections of BDNF accelerated kindling development and did not affect the level of phosphorylated TrkB receptors	(Xu et al., 2004)
	Viral vectors to locally supplement FGF-2 and BDNF	3 days after SE	Rats, in vivo, pilocarpine (300 mg/kg), interrupted after 2 hours with diazepam. SRS 2-3 weeks after SE	Reduced epileptogenesis; Reduced the occurrence of spontaneous seizures. Seizures were significantly less severe in vector-treated rats although, on average, they had the same duration in both groups; All untreated animals displayed hippocampal sclerosis and SRSs. Animals treated had significant reduction of cell loss in the hippocampus; Increased neurogenesis and reduced neuronal damage	(Paradiso et al., 2009); (Simonato and Zucchini, 2010); (Bovolenta et al., 2010)
↓	BDNF (+/-)	n.a.	Rats, in vivo, penicillin administered intra-cortically; epileptiform activity at 24-29d	Suppressed epileptiform activity; reduced levels of BDNF led to a reduction of absolute Gamma band power	(Abidin et al., 2011)
	BDNF (+/-)	n.a.	Mice, in vivo, kindling model (100 Hz frequency, 1s duration)	Greater than twofold reduction in the rate of kindling development; maintenance of kindling is unaffected	(Kokaia et al., 1995a)
	BDNF (-/-) and TrkB (-/-)	n.a.	Mice, in vivo, kindling model (frequency of 60 Hz and intensity 500 to 700A; twice daily for 11 days, at age 2-4 months)	Modest impairment of epileptogenesis and increased hippocampal TrkB activation were detected in BDNF (-/-) mice. In contrast, reductions of electrophysiological measures and no behavioral evidence of epileptogenesis were detected in TrkB(-/-) mice; Whereas TrkB can be activated, and epileptogenesis develops in BDNF(-/-) mice, the plasticity of epileptogenesis is eliminated in TrkB(-/-) mice	(He et al., 2004)
	Acute, local, intra-hippocampal infusion of TrkB-Fc	Either 3 h before pilocarpine or immediately after diazepam	Rats, In vivo, Pilocarpine model 45 mg/kg;	When seizures were induced at the time endogenous BDNF was decreased, SE-induced neuronal damage was prevented; Exogenous BDNF infused after SE increases neuronal damage; Antibodies against BDNF prevent the early decrease of TrkB after SE; Antibodies against BDNF decrease neuronal death after SE	(Unsain et al., 2009)
	Chronic, intra-cerebro-ventricular administration of TrkB-Fc	4 days before first electrical stimulation	Rats, in vivo, kindling model (frequency of 60 Hz and intensity 500 to 700A; twice daily for 11 days)	Delays kindling development, and the degree of penetration of TrkB-Fc into the hippocampus correlates with magnitude of kindling inhibition	(Binder et al., 1999)

Abbreviations: i.p.: intra-peritoneal; n.a.: not applicable

Table 2 - Studies suggesting that BDNF modulation do not inhibit epileptogenesis or increases it.

BDNF levels	Strategies to modulate BDNF levels	Schedule of BDNF modulation	Model	Main observations	References
↑	Transgenic mice overexpressing BDNF	n.a.	Mice, in vivo, injected with KA 45 mg/kg; at 8-10 weeks of age	Increased seizure severity in response to KA; Hyperexcitability in CA3 and entorhinal cortex, but not in dentate gyrus. CA1 LTP was disrupted, indicating abnormal plasticity; Models showed a significant passive avoidance deficit;	(Croll et al., 1999)
	Transgenic mice overexpressing BDNF	n.a.	Female mice, in vivo; No epileptic stimulus applied	At age 5 – 6 months, 4 of 7 BDNF-tg displayed seizures, whereas none of the seven WT females displayed seizures; Impaired working memory functions, reduced breeding efficiency, higher anxiety-like scores, high self-grooming, impaired prepulse inhibition	(Papaleo et al., 2011)
	Transgenic mice overexpressing BDNF	n.a.	Mice, in vivo; No epileptic stimulus applied	Emergence of spontaneous seizures at 6months; At 2-3months moderate MFS	(Isgor et al., 2015)
	Direct administration of BDNF to hippocampus at a stable rhythm, for 2 weeks	No data available	Rats, in vivo; No epileptic stimulus applied	Spontaneous behavioral seizures were observed in BDNF-infused rats (8/32; 25%) but not in controls (0/20; 0%); MFS occurred in 5 BDNF-treated rats but no controls; BDNF-infused rats had increased expression of NPY in hilar neurons of the dentate gyrus relative to control rats	(Scharfman et al., 2002)
	Direct administration of BDNF to dentate gyrus granule cell layer	After decapitated at the age range 30–35 days	Rats, ex vivo, electrical current pulses	Increased responses to orthodromic stimulation; Hyperexcitability evoked by repetitive stimulation, including multiple population spikes and spreading depression. The effects of BDNF were similar to those produced by bath application of KA	(Scharfman, 1997)

Abbreviations: n.a.: not applicable

5.1. Is there a possible clinical application?

So far it is not clear if the best therapeutic strategy for epilepsies is to down or up-regulate BDNF levels, or even if this might be a good approach for this disease in humans. Moreover, the clinical trials conducted so far aiming the BDNF modulation as a therapeutic strategy for other pathologies (amyotrophic lateral sclerosis and diabetic neuropathy) have shown inconclusive results (Lu et al., 2013).

In case of up-regulating BDNF levels, there is evidence that transposition of BDNF to target injury regions have demonstrated to be challenging in clinical trials. However, recent experiments showed that this problem can be circumvented by: (1) improving BDNF delivery to target zones using different administration routs such as intranasal administration (Jiang et al., 2011) and (2) using BDNF mimetics (Kingwell, 2010). Additionally, simple alternative interventions which are known to increase BDNF in the brain should might have an important role, such as physical exercise (Vaynman et al., 2004; Hopkins et al., 2011; Mattson, 2012), intellectual stimulation (Goshen et al., 2009; Cao et al., 2011), intermittent fasting (Johnson et al., 2007; Harvie et al., 2011; Mattson, 2012), antidepressants (Jiang and Salton, 2013) and electroconvulsive shocks (Mughal et al., 2011). These simple interventions might have an important role preventing epileptogenesis in genetically predisposed individuals.

Down-regulating BDNF levels, as a therapeutic strategy, has not been tried so far in clinical trials. Nevertheless there are observations that a human BDNF genetic polymorphism in which methionine (Met) substitutes for a valine (Val) amino acid impairs BDNF neural secretion (Egan et al., 2003) is associated with altered cognitive functions and predisposition to psychiatric illnesses such as schizophrenia, Alzheimer's, depression, substance-related, and eating disorders (Egan et al., 2003; Dempster et al., 2005; Gratacòs et al., 2007; Soliman et al., 2010; Verhagen et al., 2010). These observations empower the concept that down-regulating BDNF with a therapeutic purpose might have serious adverse side effects.

Although all the challenges related to the modulation of BDNF system, this therapeutic strategy should not be dismissed. Nevertheless, it is still crucial to fully understand: (1) the differences observed in the different models; (2) the appropriate therapeutic schedule for BDNF signaling modulation; (3) the signaling pathways of BDNF responsible for decreasing seizures and epilepsy; (4) and very important, the consequences of modulating BDNF actions which are involved in so many different actions in the brain.

5.2. Is there a role for the A_{2A} of adenosine receptors?

Adenosine exerts its effects through the activation of the specific G-protein-coupled receptors: A₁, A_{2A}, A_{2B}, and A₃ receptors. The adenosine A₁ receptors (A₁R) and A_{2A} receptors (A_{2A}R) are the most sensitive to adenosine and the most abundant in the central nervous system (Fredholm et al., 2011). During a seizure, extracellular adenosine levels rapidly rise and are believed to play an important role in the termination of the seizure through binding to the inhibitory adenosine A₁R (During and Spencer, 1992; Gouder et al., 2003). Studies have shown that the adenosine A₁R agonist promotes increased latency of seizure onset, decreased seizure occurrence, shorter seizure duration, and reduced mortality rate in chemical-induced seizure models (Li et al., 2013).

Nevertheless, adenosine levels rise significantly during epileptic seizures, which could activate all adenosine receptors, including the A_{2A}R. These receptors, which are known for facilitating neuronal transmission (Berman et al., 2000; Pedata et al., 2001), have also reports of anticonvulsant effects in some observations (Huber et al., 2002). One might speculate this A_{2A}R anticonvulsant effect might be related to interplay with BDNF signaling (Sebastião et al., 2011). Functionally, it has been shown that most BDNF-mediated synaptic actions, such as in synaptic plasticity, transmission and neurotransmitter release, are fully dependent on adenosine A_{2A}R activation (Diógenes et al., 2004; Pousinha et al., 2006; Fontinha et al., 2008; Tebano et al., 2008; Jerónimo-Santos et al., 2014; Rodrigues et al., 2014b)..

Nevertheless, recent observation suggest that both the A_{2A} R agonist and antagonist, CGS 21680 and ZM 241385, respectively, did not promote changes in seizure parameters, promoting a more determinant anti-seizure role of A₁R (Siebel et al., 2015)

6. Final considerations

Down regulation of BDNF signaling, including through regulation of TrkB receptors seems to be a possible clinical therapeutic strategy for epilepsy. It matters, however, to have a deep comprehension of the consequences of continuous upregulation of BDNF in animal models before any clinical trial. Targeting specific brain areas known to be the epileptic focus or targeting lower mediators in the cascade, such as CREB might reveal a promising strategy with less adverse effects. Also, deeper assessment of the influence of BDNF on MFS and NMDA neurons would help to identify possible therapeutic targets, other than interfering with the entire BDNF signaling.

It was also noted during this review that there are few studies of BDNF modulation after the epileptic model is displaying SRS, consequently it would be interesting some investigation on the anti-seizure properties of BDNF regarding the anti-seizure definition explored in this review.

Also remarkably is the large heterogeneity of variables in the studies of the effects of BDNF regulation in epileptic models. For that purpose it would be interesting to establish a protocol with standard outcomes to measure anti-epileptogenic and anti-seizure properties of BDNF as well as a standard animal and epileptic model for this analysis capable of closely reproduce human epilepsies characteristics.

References

- Abidin I et al. (2011) Penicillin induced epileptiform activity and EEG spectrum analysis of BDNF heterozygous mice: an in vivo electrophysiological study. *Brain Res Bull* 86:159–164.
- Acsády L, Kamondi A, Sik A, Freund T, Buzsáki G (1998) GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J Neurosci* 18:3386–3403.
- Anon (1981) Proposal for Revised Clinical and Electroencephalographic Classification of Epileptic Seizures. *Epilepsia* 22:489–501.
- Arévalo JC, Wu SH (2006) Neurotrophin signaling: many exciting surprises! *Cell Mol Life Sci* 63:1523–1537.
- Berkemeier LR, Winslow JW, Kaplan DR, Nikolics K, Goeddel D V., Rosenthal A (1991) Neurotrophin-5: A novel neurotrophic factor that activates trk and trkB. *Neuron* 7:857–866.
- Berkovic SF, Mulley JC, Scheffer IE, Petrou S (2006) Human epilepsies: interaction of genetic and acquired factors. *Trends Neurosci* 29:391–397.
- Berman RF, Fredholm BB, Aden U, O'Connor WT (2000) Evidence for increased dorsal hippocampal adenosine release and metabolism during pharmacologically induced seizures in rats. *Brain Res* 872:44–53.
- Binder DK, Croll SD, Gall CM, Scharfman HE (2001) BDNF and epilepsy: Too much of a good thing? *Trends Neurosci* 24:47–53.
- Binder DK, Routbort MJ, Ryan TE, Yancopoulos GD, McNamara JO (1999) Selective inhibition of kindling development by intraventricular administration of TrkB receptor body. *J Neurosci* 19:1424–1436.
- Boutillier J, Ceni C, Pagdala PC, Forgie A, Neet KE, Barker PA (2008) Proneurotrophins Require Endocytosis and Intracellular Proteolysis to Induce TrkA Activation. *J Biol Chem* 283:12709–12716.
- Bovolenta R, Zucchini S, Paradiso B, Rodi D, Merigo F, Navarro Mora G, Osculati F, Berto E, Marconi P, Marzola A, Fabene PF, Simonato M (2010) Hippocampal FGF-2 and BDNF overexpression attenuates epileptogenesis-associated neuroinflammation and reduces spontaneous recurrent seizures. *J Neuroinflammation* 7:81.
- Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA (1998) Selective changes in single cell GABAA receptor subunit expression and function in temporal lobe epilepsy. *Nat Med* 4:1166–1172.
- Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Kelly ME, Coulter DA (2001) γ -Aminobutyric acidA receptor subunit expression predicts functional changes in hippocampal dentate granule cells during postnatal development. *J Neurochem* 77:1266–1278.
- Buckmaster PS (2014) Does mossy fiber sprouting give rise to the epileptic state? *Adv Exp Med Biol* 813:161–168.
- Buckmaster PS, Lew FH (2011) Rapamycin suppresses mossy fiber sprouting but not seizure frequency in a mouse model of temporal lobe epilepsy. *J Neurosci* 31:2337–2347.
- Buckmaster PS, Zhang GF, Yamawaki R (2002) Axon sprouting in a model of temporal lobe epilepsy creates a

- predominantly excitatory feedback circuit. *J Neurosci* 22:6650–6658.
- Buhl EH, Otis TS, Mody I (1996) Zinc-Induced Collapse of Augmented Inhibition by GABA in a Temporal Lobe Epilepsy Model. *Science* (80-) 271:369–373.
- Canas N, Pereira IT, Ribeiro JA, Sebastião AM (2004) Brain-derived neurotrophic factor facilitates glutamate and inhibits GABA release from hippocampal synaptosomes through different mechanisms. *Brain Res* 1016:72–78.
- Cao L, Choi EY, Liu X, Martin A, Wang C, Xu X, During MJ (2011) White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metab* 14:324–338.
- Casaccia-Bonnet P, Carter BD, Dobrowsky RT, Chao M V (1996) Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. *Nature* 383:716–719.
- Chao M V., Hempstead BL (1995) p75 and Trk: A two-receptor system. *Trends Neurosci* 18:321–326.
- Croll SD, Suri C, Compton DL, Simmons M V, Yancopoulos GD, Lindsay RM, Wiegand SJ, Rudge JS, Scharfman HE (1999) Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and in vitro hyperexcitability in the hippocampus and entorhinal cortex. *Neuroscience* 93:1491–1506.
- da Silva FL, Blanes W, Kalitzin SN, Parra J, Suffczynski P, Velis DN (2003) Epilepsies as Dynamical Diseases of Brain Systems: Basic Models of the Transition Between Normal and Epileptic Activity. *Epilepsia* 44:72–83.
- Dechant G, Barde Y-A (2002) The neurotrophin receptor p75NTR: novel functions and implications for diseases of the nervous system. *Nat Neurosci* 5:1131–1136.
- Dempster E, Touloupoulou T, McDonald C, Bramon E, Walshe M, Filbey F, Wickham H, Sham PC, Murray RM, Collier DA (2005) Association between BDNF val66 met genotype and episodic memory. *Am J Med Genet B Neuropsychiatr Genet* 134B:73–75.
- Diógenes MJ, Fernandes CC, Sebastião AM, Ribeiro JA (2004) Activation of adenosine A2A receptor facilitates brain-derived neurotrophic factor modulation of synaptic transmission in hippocampal slices. *J Neurosci* 24:2905–2913.
- Drube W, Lessmann A, Materlik G (1994) Resonant Anomalous X-ray Scattering. North-Holland, Amsterdam.
- Dudek FE, Shao L-R (2004) Mossy fiber sprouting and recurrent excitation: direct electrophysiologic evidence and potential implications. *Epilepsy Curr* 4:184–187.
- During MJ, Spencer DD (1992) Adenosine: a potential mediator of seizure arrest and postictal refractoriness. *Ann Neurol* 32:618–624.
- Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met Polymorphism Affects Activity-Dependent Secretion of BDNF and Human Memory and Hippocampal Function. *Cell* 112:257–269.
- Eide FF, Vining ER, Eide BL, Zang K, Wang XY, Reichardt LF (1996) Naturally occurring truncated trkB receptors

- have dominant inhibitory effects on brain-derived neurotrophic factor signaling. *J Neurosci* 16:3123–3129.
- Elmer E, Kokaia M, Ernfors P, Ferencz I, Kokaia Z, Lindvall O (1997) Suppressed kindling epileptogenesis and perturbed BDNF and TrkB gene regulation in NT-3 mutant mice. *Exp Neurol* 145:93–103.
- Erickson JC, Clegg KE, Palmiter RD (1996) Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 381:415–418.
- Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J, Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Mathern GW, Moshé SL, Perucca E, Scheffer IE, Tomson T, Watanabe M, Wiebe S (2014) ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* 55:475–482.
- Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J (2005) Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 46:470–472.
- Fontinha BM, Diógenes MJ, Ribeiro JA, Sebastião AM (2008) Enhancement of long-term potentiation by brain-derived neurotrophic factor requires adenosine A2A receptor activation by endogenous adenosine. *Neuropharmacology* 54:924–933.
- Fredholm BB, IJzerman AP, Jacobson KA, Linden J, Müller CE (2011) International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update. *Pharmacol Rev* 63:1–34.
- Friedman WJ (2000) Neurotrophins induce death of hippocampal neurons via the p75 receptor. *J Neurosci* 20:6340–6346.
- Gall C, Lauterborn J, Bundman M, Murray K, Isackson P (1991) Seizures and the regulation of neurotrophic factor and neuropeptide gene expression in brain. *Epilepsy Res Suppl* 4:225–245.
- Gall CM (1993) Seizure-Induced Changes in Neurotrophin Expression: Implications for Epilepsy. *Exp Neurol* 124:150–166.
- Gall CM, Lauterborn JC, Guthrie KM, Stinis CT (1997) Seizures and the regulation of neurotrophic factor expression: associations with structural plasticity in epilepsy. *Adv Neurol* 72:9–24.
- Garcia ML, Garcia VB, Isackson PJ, Windebank AJ (1997) Long-term alterations in growth factor mRNA expression following seizures. *Neuroreport* 8:1445–1449.
- Glazner GW, Camandola S, Mattson MP (2000) Nuclear factor-kappaB mediates the cell survival-promoting action of activity-dependent neurotrophic factor peptide-9. *J Neurochem* 75:101–108.
- Goldberg EM, Coulter DA (2013) Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. *Nat Rev Neurosci* 14:337–349.
- Goshen I, Avital A, Kreisel T, Licht T, Segal M, Yirmiya R (2009) Environmental enrichment restores memory functioning in mice with impaired IL-1 signaling via reinstatement of long-term potentiation and spine size

- enlargement. *J Neurosci* 29:3395–3403.
- Gouder N, Fritschy J-M, Boison D (2003) Seizure Suppression by Adenosine A1 Receptor Activation in a Mouse Model of Pharmacoresistant Epilepsy. *Epilepsia* 44:877–885.
- Grabenstatter HL, Russek SJ, Brooks-Kayal AR (2012) Molecular pathways controlling inhibitory receptor expression. *Epilepsia* 53 Suppl 9:71–78.
- Gratacòs M, González JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X (2007) Brain-Derived Neurotrophic Factor Val66Met and Psychiatric Disorders: Meta-Analysis of Case-Control Studies Confirm Association to Substance-Related Disorders, Eating Disorders, and Schizophrenia. *Biol Psychiatry* 61:911–922.
- Grone BP, Baraban SC (2015) Animal models in epilepsy research: legacies and new directions. *Nat Neurosci* 18:339–343.
- Gutiérrez R (2002) Activity-dependent expression of simultaneous glutamatergic and GABAergic neurotransmission from the mossy fibers in vitro. *J Neurophysiol* 87:2562–2570.
- Gutiérrez R, Heinemann U (2001) Kindling induces transient fast inhibition in the dentate gyrus--CA3 projection. *Eur J Neurosci* 13:1371–1379.
- Hammer edited by SJMGD, Hammer edited by SJM (2010) 7. New York: McGraw-Hill Medical.
- Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G, Cuzick J, Jebb SA, Martin B, Cutler RG, Son TG, Maudsley S, Carlson OD, Egan JM, Flyvbjerg A, Howell A (2011) The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. *Int J Obes (Lond)* 35:714–727.
- He X-P, Kotloski R, Nef S, Luikart BW, Parada LF, McNamara JO (2004) Conditional deletion of TrkB but not BDNF prevents epileptogenesis in the kindling model. *Neuron* 43:31–42.
- Heinrich C, Lähteinen S, Suzuki F, Anne-Marie L, Huber S, Häussler U, Haas C, Larmet Y, Castren E, Depaulis A (2011) Increase in BDNF-mediated TrkB signaling promotes epileptogenesis in a mouse model of mesial temporal lobe epilepsy. *Neurobiol Dis* 42:35–47.
- Heng K, Haney MM, Buckmaster PS (2013) High-dose rapamycin blocks mossy fiber sprouting but not seizures in a mouse model of temporal lobe epilepsy. *Epilepsia* 54:1535–1541.
- Hong Z, Li W, Qu B, Zou X, Chen J, Sander JW, Zhou D (2014) Serum brain-derived neurotrophic factor levels in epilepsy. *Eur J Neurol* 21:57–64.
- Hopkins ME, Nitecki R, Bucci DJ (2011) Physical exercise during adolescence versus adulthood: differential effects on object recognition memory and brain-derived neurotrophic factor levels. *Neuroscience* 194:84–94.
- Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* 24:677–736.
- Huber A, Güttinger M, Möhler H, Boison D (2002) Seizure suppression by adenosine A(2A) receptor activation in a

- rat model of audiogenic brainstem epilepsy. *Neurosci Lett* 329:289–292.
- Isackson PJ, Huntsman MM, Murray KD, Gall CM (1991) BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: Temporal patterns of induction distinct from NGF. *Neuron* 6:937–948.
- Isgor C, Pare C, McDole B, Coombs P, Guthrie K (2015) Expansion of the dentate mossy fiber-CA3 projection in the brain-derived neurotrophic factor-enriched mouse hippocampus. *Neuroscience* 288:10–23.
- Jerónimo-Santos A, Batalha VL, Müller CE, Baqi Y, Sebastião AM, Lopes L V., Diógenes MJ (2014) Impact of in vivo chronic blockade of adenosine A2A receptors on the BDNF-mediated facilitation of LTP. *Neuropharmacology* 83:99–106.
- Jiang C, Salton SR (2013) The Role of Neurotrophins in Major Depressive Disorder. *Transl Neurosci* 4:46–58.
- Jiang Y, Wei N, Lu T, Zhu J, Xu G, Liu X (2011) Intranasal brain-derived neurotrophic factor protects brain from ischemic insult via modulating local inflammation in rats. *Neuroscience* 172:398–405.
- Johnson JB, Summer W, Cutler RG, Martin B, Hyun D-H, Dixit VD, Pearson M, Nassar M, Telljohann R, Tellejohan R, Maudsley S, Carlson O, John S, Laub DR, Mattson MP (2007) Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. *Free Radic Biol Med* 42:665–674.
- Kandratavicius L, Monteiro MR, Assirati JAJ, Carlotti CGJ, Hallak JE, Leite JP (2013) Neurotrophins in mesial temporal lobe epilepsy with and without psychiatric comorbidities. *J Neuropathol Exp Neurol* 72:1029–1042.
- Kang HJ, Schuman EM (1995) Neurotrophin-induced modulation of synaptic transmission in the adult hippocampus. *J Physiol Paris* 89:11–22.
- Kaplan DR, Martin-Zanca D, Parada LF (1991) Tyrosine phosphorylation and tyrosine kinase activity of the trk proto-oncogene product induced by NGF. *Nature* 350:158–160.
- Kiessling M, Gass P (1993) Immediate Early Gene Expression in Experimental Epilepsy. *Brain Pathol* 3:381–393.
- Kingwell K (2010) Neurodegenerative disease: BDNF copycats. *Nat Rev Drug Discov* 9:433.
- Klein R, Jing S, Nanduri V, O'Rourke E, Barbacid M (1991) The trk proto-oncogene encodes a receptor for nerve growth factor. *Cell* 65:189–197.
- Kojima N, Borlikova G, Sakamoto T, Yamada K, Ikeda T, Itohara S, Niki H, Endo S (2008) Inducible cAMP early repressor acts as a negative regulator for kindling epileptogenesis and long-term fear memory. *J Neurosci* 28:6459–6472.
- Kokaia M, Ernfors P, Kokaia Z, Elmer E, Jaenisch R, Lindvall O (1995a) Suppressed epileptogenesis in BDNF mutant mice. *Exp Neurol* 133:215–224.
- Kokaia M, Ernfors P, Kokaia Z, Elmer E, Jaenisch R, Lindvall O (1995b) Suppressed Epileptogenesis in BDNF Mutant Mice. *Exp Neurol* 133:215–224.

- Kokaia Z, Gido G, Ringstedt T, Bengzon J, Kokaia M, Siesjö BK, Persson H, Lindvall O (1993) Rapid increase of BDNF mRNA levels in cortical neurons following spreading depression: regulation by glutamatergic mechanisms independent of seizure activity. *Brain Res Mol Brain Res* 19:277–286.
- Koyama R, Ikegaya Y (2004) Mossy fiber sprouting as a potential therapeutic target for epilepsy. *Curr Neurovasc Res* 1:3–10.
- Koyama R, Yamada MK, Fujisawa S, Katoh-Semba R, Matsuki N, Ikegaya Y (2004) Brain-derived neurotrophic factor induces hyperexcitable reentrant circuits in the dentate gyrus. *J Neurosci* 24:7215–7224.
- Kuramoto S, Yasuhara T, Agari T, Kondo A, Jing M, Kikuchi Y, Shinko A, Wakamori T, Kameda M, Wang F, Kin K, Eda Hiro S, Miyoshi Y, Date I (2011) BDNF-secreting capsule exerts neuroprotective effects on epilepsy model of rats. *Brain Res* 1368:281–289.
- Lagrange AH, Botzolakis EJ, Macdonald RL (2007) Enhanced macroscopic desensitization shapes the response of $\alpha 4$ subtype-containing GABA_A receptors to synaptic and extrasynaptic GABA. *J Physiol* 578:655–676.
- Lahtinen S, Pitkanen A, Koponen E, Saarelainen T, Castren E (2003) Exacerbated status epilepticus and acute cell loss, but no changes in epileptogenesis, in mice with increased brain-derived neurotrophic factor signaling. *Neuroscience* 122:1081–1092.
- Lamballe F, Klein R, Barbacid M (1991) trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. *Cell* 66:967–979.
- Lanz TA, Bove SE, Pilsner CD, Mariga A, Drummond EM, Cadelina GW, Adamowicz WO, Swetter BJ, Carmel S, Dumin JA, Kleiman RJ (2012) Robust changes in expression of brain-derived neurotrophic factor (BDNF) mRNA and protein across the brain do not translate to detectable changes in BDNF levels in CSF or plasma. *Biomarkers Biochem Indic Expo response, susceptibility to Chem* 17:524–531.
- Lee FS, Chao M V. (2001) Activation of Trk neurotrophin receptors in the absence of neurotrophins. *Proc Natl Acad Sci* 98:3555–3560.
- Lee R, Kermani P, Teng KK, Hempstead BL (2001) Regulation of cell survival by secreted proneurotrophins. *Science* 294:1945–1948.
- Leßmann V (1998) Neurotrophin-Dependent Modulation of Glutamatergic Synaptic Transmission in the Mammalian CNS. *Gen Pharmacol Vasc Syst* 31:667–674.
- Leßmann V, Heumann R (1998) Modulation of unitary glutamatergic synapses by neurotrophin-4/5 or brain-derived neurotrophic factor in hippocampal microcultures: presynaptic enhancement depends on pre-established paired-pulse facilitation. *Neuroscience* 86:399–413.
- Levine ES, Crozier RA, Black IB, Plummer MR (1998) Brain-derived neurotrophic factor modulates hippocampal synaptic transmission by increasing N-methyl-D-aspartic acid receptor activity. *Proc Natl Acad Sci U S A* 95:10235–10239.
- Levine ES, Dreyfus CF, Black IB, Plummer MR (1995) Brain-derived neurotrophic factor rapidly enhances synaptic

- transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. *Proc Natl Acad Sci U S A* 92:8074–8077.
- Li M, Kang R, Shi J, Liu G, Zhang J (2013) Anticonvulsant activity of B2, an adenosine analog, on chemical convulsant-induced seizures. *PLoS One* 8:e67060.
- Lindvall O, Kokaia Z, Bengzon J, Elmer E, Kokaia M (1994) Neurotrophins and brain insults. *Trends Neurosci* 17:490–496.
- Lipsky RH, Xu K, Zhu D, Kelly C, Terhakopian A, Novelli A, Marini AM (2001) Nuclear factor kappaB is a critical determinant in N-methyl-D-aspartate receptor-mediated neuroprotection. *J Neurochem* 78:254–264.
- Lohof AM, Ip NY, Poo MM (1993) Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. *Nature* 363:350–353.
- Longo DL (2012) 369 Seizures and Epilepsy. McGraw-Hill.
- Lowenstein DH (2015) Seizures and Epilepsy. In: *Harrison's principles of internal medicine* 19th Ed, 19th ed., pp 2542–2675. McGraw-Hill Education.
- Lu B (2003) Pro-Region of Neurotrophins. *Neuron* 39:735–738.
- Lu B, Nagappan G, Guan X, Nathan PJ, Wren P (2013) BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat Rev Neurosci* 14:401–416.
- Lund I V, Hu Y, Raol YH, Benham RS, Faris R, Russek SJ, Brooks-Kayal AR (2008) BDNF selectively regulates GABAA receptor transcription by activation of the JAK/STAT pathway. *Sci Signal* 1:ra9.
- Marini a. M, Jiang X, Wu X, Pan H, Guo Z, Mattson MP, Blondeau N, Novelli a., Lipsky RH (2007) Preconditioning and neurotrophins: A model for brain adaptation to seizures, ischemia and other stressful stimuli. *Amino Acids* 32:299–304.
- Mathern GW, Babb TL, Micevych PE, Blanco CE, Pretorius JK (1997) Granule cell mRNA levels for BDNF, NGF, and NT-3 correlate with neuron losses or supragranular mossy fiber sprouting in the chronically damaged and epileptic human hippocampus. *Mol Chem Neuropathol* 30:53–76.
- Mattson MP (2012) Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. *Cell Metab* 16:706–722.
- Meffert MK, Chang JM, Wiltgen BJ, Fanselow MS, Baltimore D (2003) NF-kappa B functions in synaptic signaling and behavior. *Nat Neurosci* 6:1072–1078.
- Messaoudi E, Bårdsen K, Srebro B, Bramham CR (1998) Acute intrahippocampal infusion of BDNF induces lasting potentiation of synaptic transmission in the rat dentate gyrus. *J Neurophysiol* 79:496–499.
- Miklic S, Juric DM, Carman-Krzan M, Caman-Krzan M (2004) Differences in the regulation of BDNF and NGF synthesis in cultured neonatal rat astrocytes. *Int J Dev Neurosci* 22:119–130.

- Morgan JI, Curran T (1991) Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Annu Rev Neurosci* 14:421–451.
- Morimoto K, Sato K, Sato S, Yamada N, Hayabara T (1998) Time-dependent changes in neurotrophic factor mRNA expression after kindling and long-term potentiation in rats. *Brain Res Bull* 45:599–605.
- Mowla SJ, Farhadi HF, Pareek S, Atwal JK, Morris SJ, Seidah NG, Murphy RA (2001) Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *J Biol Chem* 276:12660–12666.
- Mudo G, Jiang XH, Timmusk T, Bindoni M, Belluardo N (1996) Change in neurotrophins and their receptor mRNAs in the rat forebrain after status epilepticus induced by pilocarpine. *Epilepsia* 37:198–207.
- Mughal MR, Baharani A, Chigurupati S, Son TG, Chen E, Yang P, Okun E, Arumugam T, Chan SL, Mattson MP (2011) Electroconvulsive shock ameliorates disease processes and extends survival in huntingtin mutant mice. *Hum Mol Genet* 20:659–669.
- Murray KD, Isackson PJ, Eskin TA, King MA, Montesinos SP, Abraham LA, Roper SN (2000) Altered mRNA expression for brain-derived neurotrophic factor and type II calcium/calmodulin-dependent protein kinase in the hippocampus of patients with intractable temporal lobe epilepsy. *J Comp Neurol* 418:411–422.
- Noe F, Pool A-H, Nissinen J, Gobbi M, Bland R, Rizzi M, Balducci C, Ferraguti F, Sperk G, During MJ, Pitkanen A, Vezzani A (2008) Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. *Brain* 131:1506–1515.
- Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung W-H, Hempstead BL, Lu B (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* 306:487–491.
- Papaleo F, Silverman JL, Aney J, Tian Q, Barkan CL, Chadman KK, Crawley JN (2011) Working memory deficits, increased anxiety-like traits, and seizure susceptibility in BDNF overexpressing mice. *Learn Mem* 18:534–544.
- Paradiso B, Marconi P, Zucchini S, Berto E, Binaschi A, Bozac A, Buzzi A, Mazzuferi M, Magri E, Navarro Mora G, Rodi D, Su T, Volpi I, Zanetti L, Marzola A, Manservigi R, Fabene PF, Simonato M (2009) Localized delivery of fibroblast growth factor-2 and brain-derived neurotrophic factor reduces spontaneous seizures in an epilepsy model. *Proc Natl Acad Sci U S A* 106:7191–7196.
- Pedata F, Corsi C, Melani A, Bordoni F, Latini S (2001) Adenosine extracellular brain concentrations and role of A2A receptors in ischemia. *Ann N Y Acad Sci* 939:74–84.
- Porter BE, Lund I V, Varodayan FP, Wallace RW, Blendy JA (2008) The role of transcription factors cyclic-AMP responsive element modulator (CREM) and inducible cyclic-AMP early repressor (ICER) in epileptogenesis. *Neuroscience* 152:829–836.
- Pousinha PA, Diogenes MJ, Ribeiro JA, Sebastião AM (2006) Triggering of BDNF facilitatory action on neuromuscular transmission by adenosine A2A receptors. *Neurosci Lett* 404:143–147.
- Qian Z, Gilbert ME, Colicos MA, Kandel ER, Kuhl D (1993) Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. *Nature* 361:453–457.

- Qiao X, Suri C, Knusel B, Noebels JL (2001) Absence of hippocampal mossy fiber sprouting in transgenic mice overexpressing brain-derived neurotrophic factor. *J Neurosci Res* 64:268–276.
- Quyen ML Van, Navarro V, Martinerie J, Baulac M, Varela FJ (2003) Toward a Neurodynamical Understanding of Ictogenesis. *Epilepsia* 44:30–43.
- Reibel S, Vivien-Roels B, Le BT, Larmet Y, Carnahan J, Marescaux C, Depaulis A (2000) Overexpression of neuropeptide Y induced by brain-derived neurotrophic factor in the rat hippocampus is long lasting. *Eur J Neurosci* 12:595–605.
- Roberts DS, Hu Y, Lund I V, Brooks-Kayal AR, Russek SJ (2006) Brain-derived neurotrophic factor (BDNF)-induced synthesis of early growth response factor 3 (Egr3) controls the levels of type A GABA receptor alpha 4 subunits in hippocampal neurons. *J Biol Chem* 281:29431–29435.
- Rocamora N, Palacios JM, Mengod G (1992) Limbic seizures induce a differential regulation of the expression of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3, in the rat hippocampus. *Brain Res Mol Brain Res* 13:27–33.
- Rodrigues TM, Jerónimo-Santos A, Outeiro TF, Sebastião AM, Diógenes MJ (2014a) Challenges and promises in the development of neurotrophic factor-based therapies for Parkinson's disease. *Drugs Aging* 31:239–261.
- Rodrigues TM, Jerónimo-Santos A, Sebastião AM, Diógenes MJ (2014b) Adenosine A(2A) Receptors as novel upstream regulators of BDNF-mediated attenuation of hippocampal Long-Term Depression (LTD). *Neuropharmacology* 79:389–398.
- Routbort M, Ryan T (1997) TrkB-IgG does not inhibit mossy fiber sprouting in an in vitro model. *Soc Neurosci Abstr.*
- Rudge JS, Mather PE, Pasnikowski EM, Cai N, Corcoran T, Acheson A, Anderson K, Lindsay RM, Wiegand SJ (1998) Endogenous BDNF protein is increased in adult rat hippocampus after a kainic acid induced excitotoxic insult but exogenous BDNF is not neuroprotective. *Exp Neurol* 149:398–410.
- Scharfman HE (1997) Hyperexcitability in combined entorhinal/hippocampal slices of adult rat after exposure to brain-derived neurotrophic factor. *J Neurophysiol* 78:1082–1095.
- Scharfman HE, Goodman JH, Sollas AL, Croll SD (2002) Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. *Exp Neurol* 174:201–214.
- Sebastião AM, Assaife-Lopes N, Diógenes MJ, Vaz SH, Ribeiro J a (2011) Modulation of brain-derived neurotrophic factor (BDNF) actions in the nervous system by adenosine A(2A) receptors and the role of lipid rafts. *Biochim Biophys Acta* 1808:1340–1349.
- Segal RA, Greenberg ME (1996) Intracellular signaling pathways activated by neurotrophic factors. *Annu Rev Neurosci* 19:463–489.
- Seidah NG, Benjannet S, Pareek S, Chrétien M, Murphy RA (1996) Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. *FEBS Lett* 379:247–250.

- Siebel AM, Menezes FP, Capiotti KM, Kist LW, da Costa Schaefer I, Frantz JZ, Bogo MR, Da Silva RS, Bonan CD (2015) Role of adenosine signaling on pentylentetrazole-induced seizures in zebrafish. *Zebrafish* 12:127–136.
- Simonato M (2014) Gene therapy for epilepsy. *Epilepsy Behav* 38:125–130.
- Simonato M, Zucchini S (2010) Are the neurotrophic factors a suitable therapeutic target for the prevention of epileptogenesis? *Epilepsia* 51 Suppl 3:48–51.
- Soliman F, Glatt CE, Bath KG, Levita L, Jones RM, Pattwell SS, Jing D, Tottenham N, Amso D, Somerville LH, Voss HU, Glover G, Ballon DJ, Liston C, Teslovich T, Van Kempen T, Lee FS, Casey BJ (2010) A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science* 327:863–866.
- Somjen GG (2004) *Ions in the Brain Normal Function, Seizures, and Stroke*. New York: Oxford University Press.
- Squinto SP, Stitt TN, Aldrich TH, Davis S, Blanco SM, Radziejewski C, Glass DJ, Masiakowski P, Furth ME, Valenzuela DM, Distefano PS, Yancopoulos GD (1991) trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. *Cell* 65:885–893.
- Suen PC, Wu K, Levine ES, Mount HT, Xu JL, Lin SY, Black IB (1997) Brain-derived neurotrophic factor rapidly enhances phosphorylation of the postsynaptic N-methyl-D-aspartate receptor subunit 1. *Proc Natl Acad Sci U S A* 94:8191–8195.
- Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L (1989) Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol* 26:321–330.
- Suzuki F, Junier MP, Guilhem D, Sorensen JC, Onteniente B (1995) Morphogenetic effect of kainate on adult hippocampal neurons associated with a prolonged expression of brain-derived neurotrophic factor. *Neuroscience* 64:665–674.
- Swiech L, Perycz M, Malik A, Jaworski J (2008) Role of mTOR in physiology and pathology of the nervous system. *Biochim Biophys Acta* 1784:116–132.
- Takahashi M, Hayashi S, Kakita A, Wakabayashi K, Fukuda M, Kameyama S, Tanaka R, Takahashi H, Nawa H (1999) Patients with temporal lobe epilepsy show an increase in brain-derived neurotrophic factor protein and its correlation with neuropeptide Y. *Brain Res* 818:579–582.
- Tanaka T, Saito H, Matsuki N (1997) Inhibition of GABAA synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. *J Neurosci* 17:2959–2966.
- Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME (1998) Ca²⁺ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron* 20:709–726.
- Tebano MT, Martire A, Potenza RL, Grò C, Pepponi R, Armida M, Domenici MR, Schwarzschild MA, Chen JF, Popoli P (2008) Adenosine A(2A) receptors are required for normal BDNF levels and BDNF-induced potentiation of synaptic transmission in the mouse hippocampus. *J Neurochem* 104:279–286.
- Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, Kermani P, Torkin R, Chen Z-Y, Lee FS, Kraemer RT,

- Nykjaer A, Hempstead BL (2005) ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J Neurosci* 25:5455–5463.
- Teng KK, Felice S, Kim T, Hempstead BL (2010) Understanding proneurotrophin actions: Recent advances and challenges. *Dev Neurobiol* 70:350–359.
- Thoenen H (1995) Neurotrophins and Neuronal Plasticity. *Science* (80-) 270:593–598.
- Unsain N, Montroull LE, Masco DH (2009) Brain-derived neurotrophic factor facilitates TrkB down-regulation and neuronal injury after status epilepticus in the rat hippocampus. *J Neurochem* 111:428–440.
- Urfer R, Tsoulfas P, O’Connell L, Shelton DL, Parada LF, Presta LG (1995) An immunoglobulin-like domain determines the specificity of neurotrophin receptors. *EMBO J* 14:2795–2805.
- Vaidya VA, Siuciak JA, Du F, Duman RS (1999) Hippocampal mossy fiber sprouting induced by chronic electroconvulsive seizures. *Neuroscience* 89:157–166.
- Vaynman S, Ying Z, Gomez-Pinilla F (2004) Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *Eur J Neurosci* 20:2580–2590.
- Vaz SH, Cristóvão-Ferreira S, Ribeiro JA, Sebastião AM (2008) Brain-derived neurotrophic factor inhibits GABA uptake by the rat hippocampal nerve terminals. *Brain Res* 1219:19–25.
- Vaz SH, Jørgensen TN, Cristóvão-Ferreira S, Duflot S, Ribeiro JA, Gether U, Sebastião AM (2011) Brain-derived neurotrophic factor (BDNF) enhances GABA transport by modulating the trafficking of GABA transporter-1 (GAT-1) from the plasma membrane of rat cortical astrocytes. *J Biol Chem* 286:40464–40476.
- Verhagen M, van der Meij A, van Deurzen PAM, Janzing JGE, Arias-Vásquez A, Buitelaar JK, Franke B (2010) Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. *Mol Psychiatry* 15:260–271.
- Vezzani A, Michalkiewicz M, Michalkiewicz T, Moneta D, Ravizza T, Richichi C, Aliprandi M, Mulé F, Pirona L, Gobbi M, Schwarzer C, Sperk G (2002) Seizure susceptibility and epileptogenesis are decreased in transgenic rats overexpressing neuropeptide Y. *Neuroscience* 110:237–243.
- Vezzani A, Ravizza T, Moneta D, Conti M, Borroni A, Rizzi M, Samanin R, Maj R (1999) Brain-derived neurotrophic factor immunoreactivity in the limbic system of rats after acute seizures and during spontaneous convulsions: temporal evolution of changes as compared to neuropeptide Y. *Neuroscience* 90:1445–1461.
- Villemure de TN (1996) Epilepsy in patients with central nervous system tumors. *Curr Opin Neurol* 9:424–428.
- Walczak A, Szczepankiewicz AA, Ruszczycki B, Magalska A, Zamlynska K, Dzwonek J, Wilczek E, Zybura-Broda K, Rylski M, Malinowska M, Dabrowski M, Szczepinska T, Pawlowski K, Pyskaty M, Wlodarczyk J, Szczerbal I, Switonski M, Cremer M, Wilczynski GM (2013) Novel higher-order epigenetic regulation of the Bdnf gene upon seizures. *J Neurosci* 33:2507–2511.
- Wieser H-G (2004) ILAE Commission Report. Mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia*

- Wyneken U, Marengo JJ, Villanueva S, Soto D, Sandoval R, Gundelfinger ED, Orrego F (2003) Epilepsy-induced changes in signaling systems of human and rat postsynaptic densities. *Epilepsia* 44:243–246.
- Xie W, Song Y-J, Li D, Pan L-P, Wu Q-J, Tian X (2014) The suppression of epileptiform discharges in cultured hippocampal neurons is regulated via alterations in full-length tropomyosin-related kinase type B receptors signalling activity. *Eur J Neurosci* 40:2564–2575.
- Xu B, Michalski B, Racine R., Fahnstock M (2004) The effects of brain-derived neurotrophic factor (BDNF) administration on kindling induction, Trk expression and seizure-related morphological changes. *Neuroscience* 126:521–531.
- Zhou H, Li XM, Meinkoth J, Pittman RN (2000) Akt regulates cell survival and apoptosis at a postmitochondrial level. *J Cell Biol* 151:483–494.

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